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IL-12 Indirectly Enhances Proliferation of 5-FU-Treated Human Hematopoietic Peripheral Blood CD34$^+$ Cells

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Interleukin-12 (IL-12) or natural killer cell stimulatory factor (NKSF), has multiple effects on T lymphocytes and natural killer cells. In this study, the effect of IL-12 on human hematopoiesis was studied by analyzing the growth of CD34$^+$ peripheral blood stem cells (PBSC), in steady state. In the presence of Epo, IL-12 alone or in combination with IL-3 or SCF had no effect on the formation of colonies from CD34$^+$ cells. In culture with Epo, G-CSF, and IL-3, the effect of Flt3-ligand (FL) on CD34$^+$ PBSC was investigated in the presence or absence of IL-12. No additional effect of IL-12 was observed when combined with FL. We evaluated 5-FU-treated human CD34$^+$ PBSC proliferation in cultures with Epo, G-CSF, and IL-3, in the presence or absence of IL-12. No cytokine combination enhanced colony formation from 5-FU-treated CD34$^+$ cells. However, in cultures of 5-FU-treated human CD34$^+$ cells, the most efficient combination was IL-3 + Epo + G-CSF + Accessory cells (CD34$^-$). Furthermore, IL-12 enhanced this colony formation significantly. To investigate whether immature CD34$^+$ cells were responsible for FL or SCF, 5-FU-treated human CD34$^+$ cells were cultured with or without IL-12. Whereas no synergistic effect was observed in combination with IL-12, SCF alone significantly enhanced colony formation. However, the colony number was found to be smaller than with the potent combination of accessory cells in the presence of IL-12. These results indicate that accessory cells, lost in CD34$^+$ cell purification, could be partly responsible for an IL-12 effect on immature human PBSC proliferation. Am. J. Hematol. 58:183–188, 1998. © 1998 Wiley-Liss, Inc.

Key words: 5-FU; hematopoiesis; human; IL-12; peripheral blood

INTRODUCTION

Natural killer cell stimulatory factor (NKSF) or interleukin-12 (IL-12) was originally identified in the supernatant fluid of human Epstein-Barr (EB) virus-transformed B lymphoblastoid cell lines [1]. IL-12 is produced by peripheral blood mononuclear cells [2]. This cytokine was found to stimulate mature T lymphocytes and NK cells [3]. IL-12 is a disulfide-linked heterodimeric cytokine composed of two unrelated subunits, a larger 40-kDa (p40) and a smaller 35-kDa (p35) subunit [4]. Previous studies have shown an amino acid sequence homology between p40 and extracellular domain of IL-6 receptor [5], and among p35, IL-6, granulocyte colony-stimulating factor (G-CSF), and chicken myelomonocytic growth factor. The homology between IL-12 and ligands and receptors of cytokines was shown to stimulate hematopoietic progenitors, suggesting that IL-12 might affect the growth of hematopoietic progenitor cells.

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Recent studies have demonstrated that IL-12 may be active at early stages of murine lymphohematopoiesis [6]. The proliferation of primitive hematopoietic progenitors appears to be regulated by synergistic interactions among several cytokines. Synergistic effects of IL-12, IL-3, and an unknown serum factor have been described in primitive mobilized human hematopoietic progenitor CD34$^+$33$^-$ cells [7]. It is generally accepted that imma-

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ture hematopoietic stem cells are cell-cycle dormant. 5-Fluorouracil (5-FU) spares hematopoietic stem cells responsible for long-term repopulation [8,9].

To our knowledge, no study has been reported, on the effect of IL-12, on $CD34^+$ peripheral blood stem cells (PBSC) collected in steady state, in human healthy donors. Moreover, the effects of IL-12 on human hematopoietic progenitor 5-FU-treated $CD34^+$ cells have not been tested yet.

MATERIALS AND METHODS

Cells

Normal peripheral blood (PB) cells were obtained from adult volunteers after informed consent. Cells were separated on a Ficoll-Hypaque gradient (specific gravity: 1.077 g/ml (Eurobio, Paris, France), washed twice with RPMI supplemented with 1% L-glutamine and 1% penicillin-streptomycin (Gibco, Cergy-Pontoise, France) and then depleted of adherent cells by incubation in 75 cm^2 plastic flasks (Costar, Dütscher, Brumath, France) 2 hr in IMDM (Iscove Modified Dulbecco's Medium) (Gibco) containing 10% fetal calf serum (FCS) (Biowhittaker, Walkersville, MD). The isolation of hematopoietic progenitor cells is obtained through a positive selection of CD34 expressing cells (Myltenyi Biotec, Tebu, Le Perray-en-Yvelines, France). $CD34^+$ hematopoietic progenitor cells are indirectly magnetically labeled using a hapten-conjugated primary monoclonal antibody and an anti-hapten antibody coupled to microbeads. The magnetically labeled cells are enriched on positive selection columns in the magnetic field. The effluent, or negative fraction, contains non-adherent accessory cells ($CD34^-$).

Cytokines

RhIL-12 and rhEpo were purchased from R&D Systems (Barton Lane, Abingdon, UK) and rhIL-3 (2×10^7 UI/mg) and Flt3-ligand (FL) from Tebu. RhG-CSF glycosylated (33.6×10^6 UI/263 μg) was obtained from Roger Bellon (Chugai-Rhône-Poulenc, Neuilly sur Seine, France). RhSCF was kindly supplied by Amgen (Thousand Oaks, CA).

5-FU Treatment

$CD34^+$ cells were suspended in IMDM and supplemented with 10% FCS, penicillin, and streptomycin (1% each). The $CD34^+$ cells and 25 μg/ml 5-FU (Roche, Neuilly sur Seine, France) were incubated for 24 hr at 37°C in 25 cm^2 plastic flasks (Costar, Dutscher, Brumath, France). The cells were washed three times with IMDM supplemented with 20% FCS.

Colony Assay

Human peripheral blood $CD34^+$ cells (5×10^3 cells/ml) were separated as previously described, and plated in duplicates, in 35-mm petri dishes (Poly Labo, Strasbourg, France) at a final volume of 1 ml per dish in IMDM medium containing 0.8% methylcellulose (Fluka, Methcell, Mulhouse, France), 15% FCS, 15% human plasma, 5×10^{-5} mol/l 2-β mercaptoethanol (Sigma, Saint Quentin, France) and rhEpo at 1 UI/ml. Cultures were incubated at 37°C in a 5% CO_2 humidified incubator for 14 days and scored for BFU-E and CFU-GM colonies. All growth factors were used at predetermined optimal concentrations: rhEpo, 20 UI/ml; rhIL-3, 20 UI/ml; rhIL-12, 10 ng/ml; rhG-CSF, 100 UI/ml; rhSCF, 100 ng/ml; rhFL, 200 ng/ml.

Statistical Methods

The results expressed as the mean ± SD were obtained for four or more separate experiments. Statistical significance was determined using the Student's *t*-test.

RESULTS

Effect of rhIL-12 on Untreated PBSC Growth

To test the possibility of IL-12 effect on the proliferation of human progenitors, 5×10^3 $CD34^+$ were cultured in methylcellulose with Epo, in the presence or absence of IL-12, and in combination with other cytokines. As observed in previous studies, IL-12 alone did not stimulate any colony formation (Fig. 1). Furthermore, IL-12 did not affect Epo + IL-3 or Epo + SCF or Epo + SCF + IL-3-induced colony formation. The number of colony-forming units for granulocyte-macrophage (CFU-GM) and burst-forming units for erythroid (BFU-E) from 5×10^3 $CD34^+$ was not influenced by the addition of IL-12.

Effects of rhFlt3-Ligand on Untreated PBSC Growth

To investigate whether more committed $CD34^+$ cells were also responsive to FL, PB $CD34^+$ cells were cultured in Epo, IL-3, G-CSF, and FL (200 ng/ml), in the absence or presence of IL-12 (Table I). FL did not synergize with Epo, IL-3, and G-CSF. No additional effect of IL-12 was observed when combined with FL.

Effects of rhIL-12 on 5-FU-Treated PBSC Growth

To investigate whether IL-12 could directly affect the proliferation of immature hematopoietic stem cells, 5-FU-treated $CD34^+$ cells were cultured in methylcellulose in the presence of Epo, IL-3, and G-CSF with or without IL-12 (Table II). Results are expressed in absolute numbers of colonies per plated cells and in percentage ± SD. A small number of colonies was observed with any combination of cytokines (0.33 ± 0.25). Furthermore, the addition of IL-12 exerts a significant inhibitory effect on colony formation from $CD34^+$ alone (0.17 ± 0.14).

To determine colony formation from accessory cells, $CD34^-$ were plated under the same conditions. The colony formation from accessory cells ($CD34^-$) with or

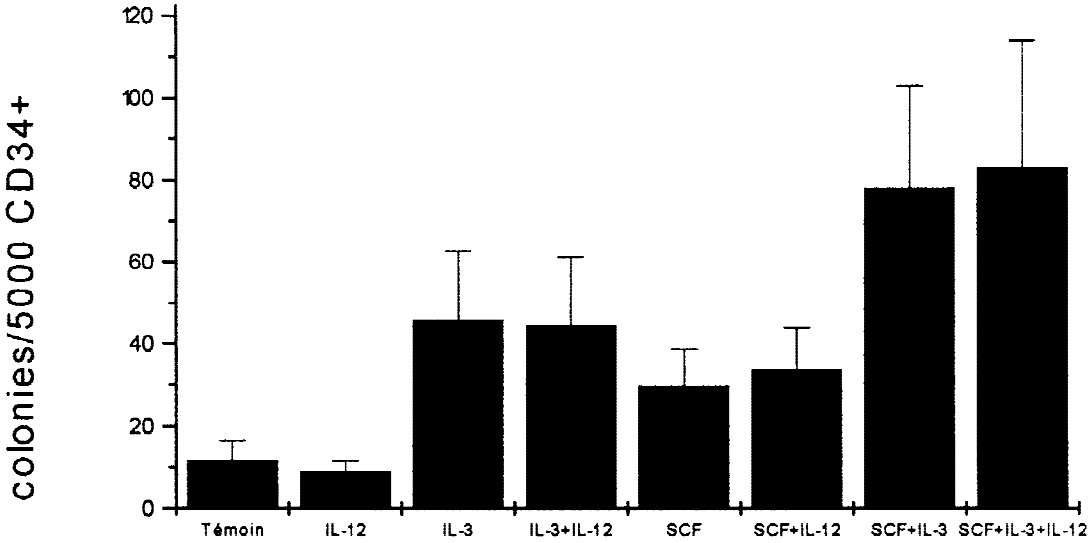

Fig. 1. Effects of IL-3 and SCF, in the absence or presence of IL-12 on colony formation. Peripheral blood CD34+ were isolated and plated at 5×10^3 cells in semisolid medium with Epo (1 UI/ml), as described in Materials and Methods. Cultures were duplicated, and supplemented with predetermined optimal concentrations of cytokines (see Materials and Methods) as indicated. Colony formation was scored after 14 days of incubation at 37°C, in 5% CO_2, in air. Results represent the mean ± SD of eight separate experiments.

without IL-12, shows that there are still some committed progenitors (respectively 0.22 ± 0.12 and 0.26 ± 0.18). Since these $CD34^+$ cells are more mature, this may explain the lack of difference of colony number from accessory cells, in the presence or absence of IL-12. However, we can note that the number of plated accessory cells was five times greater than the number of $CD34^+$ cells and that accessory cells were not 5-FU treated.

However, when accessory cells ($CD34^-$) were added to the culture medium (proportion of 5 accessory cells for 1 5-FU-treated $CD34^+$ cell), a significant enhancement of colony formation was observed (1.92 ± 0.45). Furthermore, IL-12 enhanced this colony formation over 200%. Results are expressed in percentages, 100% being by convention the number of the colony observed from 5-FU-treated $CD34^+$ cells in the presence of accessory cells. The number of colonies supported by $CD34^+$ and accessory cells in the absence or presence of IL-12 (respectively, 100% and 200 ± 33%), is far greater than the addition of the number of colonies from $CD34^+$ (17.2 ± 13% and 8.8 ± 7.3%) and the number of colonies from accessory cells (13.5 ± 9.3% and 11.4 ± 6.2%), respectively, in the absence or presence of IL-12 (30.7 ± 22.3% and 20.2 ± 13.5%). The difference observed between with or without IL-12 is significant despite the large SD, because of the large number of experiments done.

These studies demonstrated that there were synergistic effects of IL-12 on both BFU-E and CFU-GM colony formation (Table III). IL-12 significantly increased the number of both BFU-E and CFU-GM formed in response to the effective combination of IL-3 + Epo + G-CSF + non-adherent accessory cells (respectively, 3.64 ± 0.45 and 0.59 ± 0.19 in the presence of IL-12 vs. 1.78 ± 0.4 and 0.14 ± 0.05, in the absence of IL-12).

Effects of rhFlt3-Ligand or SCF on 5-FU-Treated PBSC Growth

To investigate whether more immature $CD34^+$ cells were also responsive to FL or SCF, 5-FU-treated peripheral blood $CD34^+$ were cultured in Epo, IL-3, G-CSF, and FL (200 ng/ml) or SCF (100 ng/ml), in the absence or presence of IL-12 (Table IV). In these experiments, we also examined the ability of FL and SCF to enhance colony formation from immature PBSC. These studies demonstrate that FL has no effect on Epo, IL-3, and G-CSF induced-colony formation from immature PBSC (0.25 ± 0.23). No significant synergistic enhancement of FL was observed on IL-12-induced colony formation (1.16 ± 1.06). The addition of SCF to Epo, IL-3, and G-CSF induced significantly colony formation (2.87 ± 0.9), whereas no synergistic interaction was observed between IL-12 and SCF (2.91 ± 1.1). However, this enhancement is less than with the effective combination of IL-3 + Epo + G-CSF + non-adherent accessory cells in the presence of IL-12 (6.75 ± 1.87).

DISCUSSION

IL-12 plays a key role in the regulation of immune and inflammatory responses. IL-12 stimulates the proliferation of NK and T cells and induces cytotoxicity of the same cells [1,3,10]. A number of studies have now shown the ability of IL-12 to stimulate the growth of

TABLE I. Effect of IL-12 Alone or in Combination With Flt3-Ligand on Colony Formation From Peripheral Blood CD34$^+$ Cells*

	Colony number					
	BFU-E		CFU-GM		CFU-MIX	
	− IL-12	+ IL-12	− IL-12	+ IL-12	− IL-12	+ IL-12
CD34$^+$	10.5 ± 3.6	11.5 ± 2.9	2.16 ± 1	2.5 ± 1.5	0.16 ± 0.1	0.16 ± 0.1
CD34$^+$ + Flt3-ligand	16 ± 5.7	11.5 ± 4	3.66 ± 1.2	1.33 ± 0.3	0.16 ± 0.1	0.33 ± 0.2

*5×10^3 CD34$^+$ PB cells were plated in duplicates in semisolid medium containing Epo (1 UI/ml), IL-3 (20 UI/ml), and G-CSF (100 UI/ml), with IL-12 (10 ng/ml) alone or in combination with Flt3-ligand (200 ng/ml), as previously described in Materials and Methods. Colony formation was scored after 14 days of incubation at 37°C, in 5% CO_2. Results shown are the mean ± SD of four separate experiments.

TABLE II. Effect of IL-12 Alone or in Combination With Accessory Cells on Colony Formation From 5-FU-Treated Peripheral Blood CD34$^+$ Cells†

	Colony number/ plated cells	Percentage
5-FU-treated CD34$^+$ cells	$0.33 \pm 0.25/20 \times 10^3$	17.2 ± 13*
5-FU-treated CD 34$^+$ cells + IL-12	$0.17 \pm 0.14/20 \times 10^3$	8.8 ± 7.3*
Accessory cells alone	$0.26 \pm 0.18/100 \times 10^3$	13.5 ± 9.3
Accessory cells alone + IL-12	$0.22 \pm 0.12/100 \times 10^3$	11.4 ± 6.2
5-FU-treated CD 34$^+$ cells + accessory cells	$1.92 \pm 0.45/120 \times 10^3$	100**
5-FU-treated CD 34$^+$ cells + accessory cells + IL-12	$4.23 \pm 0.64/120 \times 10^3$	220.3 ± 33**

†5-FU-treated peripheral blood CD34$^+$ cells were plated, in duplicates, in semisolid medium containing Epo (1 UI/ml), IL-3 (20 UI/ml), and G-CSF (100 UI/ml), with IL-12 (10 ng/ml) alone or in combination with non-adherent accessory cells (CD34$^-$), as previously described in Materials and Methods. Colony formation was scored after 14 days of incubation at 37°C, in 5% CO_2. Results shown are the mean and percentage ± SD of 18 separate experiments. Percentages were calculated from the number of colonies from 5-FU-treated CD34$^+$ cells + accessory cells (= 100%).

*Difference in the number of colonies supported by IL-12 vs. without IL-12 is significant at $P < 0.05$ by Student's *t*-test.

**Difference in the number of colonies supported by accessory cells + IL-12 vs. accessory cells alone is significant at $P < 0.001$ by Student's *t*-test.

hematopoietic progenitor cells but the data are complex and are sometimes conflicting.

Murine Models

Using murine models, Jacobsen et al. have indicated that IL-12 alone has no ability to stimulate the growth of primitive murine Lin$^-$Sca-1$^+$ bone marrow progenitor cells but that the addition of IL-12 to SCF and IL-3 leads to an additive enhancement of the colony [11]. Moreover, Jacobsen et al. have shown that IL-12 potently enhances the Flt3-ligand-stimulated proliferation of Lin$^-$Sca-1$^+$ progenitor murine [12].

Dybedal et al. demonstrated that IL-12 had no ability to synergize with Epo alone to stimulate the growth of erythroid progenitor cells in Lin$^-$ murine bone marrow cells. However, in the same study, it was found that IL-12 enhanced BFU-E colony formation in response to Epo + IL-4 and Epo + SCF [13].

Ploemacher et al. have used primitive bone marrow progenitor cells from 5-FU-treated mice to show that IL-12 could enhance the growth of primed stem cells in combination with IL-3, SCF, and IL-11, alone or together [14,15].

Similar studies on Lin$^-$Sca-1$^+$ cells from 5-FU-treated mice also demonstrated the ability of IL-12 to enhance SCF-induced progenitor cell growth, suggesting an effect on multipotent progenitor cells [6].

Jackson et al. have shown that IL-12 has a significant in vivo hematopoietic stimulating activity that includes the enhancement of peripheral hematopoiesis and the mobilization of murine progenitor cells to peripheral circulation [16].

These studies suggest that IL-12 could stimulate the growth of primitive multipotent and committed progenitor cells in murine bone marrow. IL-12 appears to be a potent synergistic factor for the growth of primitive murine bone marrow progenitors.

Human Models

In humans, only a few studies have shown that IL-12 can stimulate the growth of human hematopoietic progenitor cells in vitro. Bellone and Trinchieri have isolated hematopoietic progenitor cells from human bone marrow or non-mobilized peripheral blood. This isolation was based on their lack of expression of lineage specific markers. In these cells, IL-12 enhanced the formation of CFU-GM, BFU-E, and CFU-GEMM colonies when combined with SCF + IL-3. The population of progenitors used in these studies was highly heterogeneous, predominantly containing more committed progenitors, but the colony formation suggested an effect on primitive progenitors [17].

Fardoun-Joalland et al. have explored the effects of IL-12 on human PB progenitor cells depleted of adherent, CD2$^+$, and CD56$^+$ cells. These studies found no colony-stimulating activity of IL-12 alone. However, the synergistic effect of IL-12 and IL-3 was found to enhance the growth of both BFU-E and CFU-GM colony formation [18].

Hirao et al. investigated the effects of IL-12 on the growth of CD34$^+$ CD33$^-$ human progenitor mobilized

TABLE III. Effect of IL-12 With or Without Accessory Cells on Colony Formation From 5-FU-Treated Peripheral Blood CD34$^+$ Cells†

	Colony number			
	BFU-E		CFU-GM	
	− IL-12	+ IL-12	− IL-12	+ IL-12
5-FU-treated CD34$^+$	0.28 ± 0.2*	0.17 ± 0.14*	0.05 ± 0.05**	0**
5-FU-treated CD34$^+$ + Accessory cells	1.78 ± 0.4***	3.64 ± 0.45***	0.14 ± 0.05***	0.59 ± 0.19***

†5-FU-treated peripheral blood CD34$^+$ cells were plated, in duplicates, in semisolid medium containing Epo (1 UI/ml), IL-3 (20 UI/ml), and G-CSF (100 UI/ml), with IL-12 (10 ng/ml) alone or in combination with non-adherent accessory cells (CD34$^-$), as previously described in Materials and Methods. Colony formation was scored after 14 days of incubation at 37°C, in 5% CO_2. Results shown are the mean ± SD of 18 separate experiments.

*Difference in the number of BFU-E from 5-FU-treated CD34$^+$ cells supported by IL-12 vs. without IL-12 is significant at $P < 0.05$ by Student's *t*-test.

**Difference in the number of CFU-GM supported by IL-12 vs. without IL-12, from 5-FU-treated CD34$^+$ cells, is significant at $P < 0.001$ by Student's *t*-test.

***Difference in the number of CFU-GM and BFU-E from 5-FU-treated CD34$^+$ cells in presence of accessory cells, supported by IL-12 vs. in absence of IL-12 is significant at $P < 0.001$ by Student's *t*-test.

TABLE IV. Effect of IL-12 With or Without Flt3-Ligand, SCF, and Accessory Cells on Colony Formation From 5-FU-Treated Peripheral Blood CD34$^+$ Cells†

	Colony number/ plated cells	Percentage
5-FU-treated CD34$^+$ cells	$0.25 \pm 0.23/20 \times 10^3$	7.1 ± 6.5*
5-FU-treated CD34$^+$ cells + IL-12	$0/20 \times 10^3$	0
Accessory cells	$0.21 \pm 0.18/100 \times 10^3$	6 ± 5.1
Accessory cells + IL-12	$0.19 \pm 0.17/100 \times 10^3$	5.4 ± 4.8
5-FU-treated CD34$^+$ cells + Accessory cells	$3.5 \pm 1.6/120 \times 10^3$	100**
5-FU-treated CD34$^+$ cells + Accessory cells + IL-12	$6.75 \pm 1.87/120 \times 10^3$	192.8 ± 53**
5-FU-treated CD34$^+$ cells + Flt3-ligand	$0.25 \pm 0.23/20 \times 10^3$	7.1 ± 6.5
5-FU-treated CD34$^+$ cells + Flt3-ligand + IL-12	$1.16 \pm 1.06/20 \times 10^3$	33.1 ± 30
5-FU-treated CD34$^+$ cells + SCF	$2.87 \pm 0.9/20 \times 10^3$	82 ± 25.7
5-FU-treated CD34$^+$ cells + SCF + IL-12	$2.91 \pm 1.1/20 \times 10^3$	83 ± 31.7**

†5-FU-treated CD34$^+$ cells were plated, in duplicates, in semisolid medium containing Epo, IL-3, and G-CSF, with IL-12 alone or in combination with Flt3-ligand, SCF, or non-adherent accessory cells (CD34), as previously described in Materials and Methods. Results shown are the mean and percentage ± SD of four separate experiments.

*Difference in the number of colonies supported by SCF vs. CD34$^+$ alone is significant at $P < 0.05$ by Student's *t*-test.

**Difference in the number of colonies supported by IL-12 + accessory cells vs. accessory cells alone is significant at $P < 0.05$ by Student's *t*-test.

PB cells. In serum-containing cultures, IL-12 enhanced the growth of CFU-GM, but not BFU-E, when combined with IL-3 and Epo or SCF and IL-3, whereas no effect was observed either on BFU-E or CFU-GM in serum-depleted cultures, suggesting the requirement of an undefined serum factor in the synergy between IL-12 and IL-3, or IL-3 and SCF [7].

Data collected in the present study confirm previous findings that show a lack of a direct effect of IL-12 on both 5-FU-treated and untreated progenitor cells. IL-12, in steady state, does not enhance proliferation of human peripheral blood hematopoietic progenitors CD34$^+$, in combination with Epo, IL-3, or SCF.

Moreover, synergy between IL-12 and Flt3-ligand, described by Jacobsen et al. on murine Lin$^-$Sca-1$^+$ progenitors, was not found [12]. Our results show a lack of synergistic effect of Flt3-ligand on IL-12-induced colony formation from both human untreated and 5-FU-treated CD34$^-$ PBSC. These conflicting results may be due to the type of plated stem cells.

Long-term cultures have revealed that 5-FU-treated cells consisted of primitive cell populations with a self-renewal potential. Based on this data, 5-FU-treated CD34$^+$ cells were purified from peripheral blood of healthy adult donors. Simple purification procedures and 5-FU-treatment were combined to yield cells with a reasonable phenotypic purity. Based on this source of highly purified primitive cells, the effect of cytokines secreted from contaminating accessory cells, which may affect the final results, could be neglected. Previous reports suggest that IL-12 supports the colony formation from dormant murine unselected hematopoietic progenitors [6].

Bertolini et al. have evaluated G-CSF-mobilized progenitor cell CD34$^+$ proliferation in the presence or absence of IL-12. These results indicate that IL-12 did not enhance expansion of progenitors and that accessory cells lost in CD34$^+$ cell purification could be partly responsible for an IL-12 effect on progenitor cell proliferation [19].

The data collected in the present study are the first to be published regarding the effects of IL-12 on 5-FU-treated human CD34$^+$ cells, in steady state. The results indicate that IL-12 does not enhance the proliferation of 5-FU-treated CD34$^+$ human PBSC, in combination with Epo, IL-3, and G-CSF. Moreover, when combined with accessory cells (CD34$^-$), IL-12 significantly increases colony number from 5-FU-treated human CD34$^+$ PBSC,

in steady state. Thus, the ability of IL-12 to enhance colony formation appears to be indirectly mediated on immature $CD34^+$ cells. We investigated whether SCF or FL reproduces the effect of IL-12. SCF significantly induces colony formation from both untreated and primitive $CD34^+$ cells, but there is no synergy between IL-12 and SCF. Our results show a nonsignificant positive effect of Flt3-ligand on an IL-12-induced colony formation from human 5-FU treated $CD34^+$ PBSC, whereas no effect was detectable on untreated $CD34^+$ cells. Both SCF and FL/IL-12-induced colony numbers are smaller than the potent combination of IL-12 in the presence of accessory cells. Our study presents evidence that IL-12 plays a key role in early hematopoiesis. However, the mechanisms involved in the positive effect of IL-12 are complex and require the participation of at least one blood cell population, probably NK cells, T and/or B lymphocytes. The mechanism(s) of action explaining the potent ability of IL-12 to stimulate colony formation via non-adherent accessory cells ($CD34^-$) remains undetermined. IL-12 might partly act by up-regulating the receptor cell surface expression for accessory cell-secreted cytokine. Other synergistic hematopoietic growth factors appear to have the ability to up-regulate the receptor expression of other cytokine on hematopoietic progenitors. On another hand, some of our results suggest that IL-12 might act by stimulating accessory cells to secondary cytokine production [20]. The enhancing effect of IL-12 may be mediated indirectly on 5-FU-treated human peripheral $CD34^+$ cells.

The present data suggest that IL-12 may be a major stimulatory factor that could affect early human hematopoiesis.

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REFERENCES

1. Kobayashi M, Fitz L, Ryan M, Hewick RM, Clark SC, Chan S, Loudon R, Sherman F, Perussia B, Trinchieri G: Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. J Exp Med 70:827, 1989.
2. D'Andrea A, Rengaraju M, Valiante NM, Chehimi J, Kubin M, Aste M, Chan SH, Kobayashi M, Young D, Nickbarg E, Chizzonite R, Wolf SF, Trinchieri G: Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. J Exp Med 176:1387, 1992.
3. Gately MK, Desai BB, Wolitzky AG, Quinn PM, Dwyer CM, Podlaski FJ, Familetti PC, Sinigaglia F, Chizzonite R, Gubler U, Stern AS: Regulation of human lymphocyte proliferation by a heterodimeric cytokine, IL-12 (cytotoxic lymphocyte maturation factor). J Immunol 147:874, 1991.
4. Wolf SF, Temple PA, Kobayashi M, Young D, Dicig M, Lowe L, Dzialo R, Fitz L, Ferenz C, Hewick RM, Kelleher K, Herrmann SH, Clark SC, Azzoni L, Chan SH, Trinchieri G, Perussia B: Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. J Immunol 146:3074, 1991.
5. Gearing DP, Cosman D: Homology of the p40 subunit of natural killer cell stimulatory factor (NKSF) with the extracellular domain of the interleukin-6 receptor. Cell 66:9, 1991.
6. Hirayama F, Katayama N, Neben S, Donaldson D, Nickbarg EB, Clark SC, Ogawa M: Synergistic interaction between interleukin-12 and steel factor in support of proliferation of murine lymphohematopoietic progenitors in culture. Blood 83:92, 1994.
7. Hirao A, Takaue Y, Kawano Y, Sato J, Suzue T, Abe T, Saito SI, Kawahito M, Okamoto Y, Makimoto A, Kuroda Y: Synergism of interleukin 12, interleukin 3 and serum factor on primitive human hematopoietic progenitor cells. Stem Cells 13:47, 1995.
8. Hodgson GS, Bradley TR: Properties of haematopoietic stem cells surviving 5-fluorouracil treatment: Evidence for a pre-CFU-S cell? Nature 281:381, 1979.
9. Lerner C, Harrisson DE: 5-fluorouracil spares hematopoietic stem cells responsible for long-term repopulation. Exp Hematol 18:114, 1990.
10. Wolf SF, Sieburth D, Sypek J: Interleukin 12: A key modulator of immune function. Stem Cells 12:154, 1994.
11. Jacobsen SEW, Veiby OP, Smeland EB: Cytotoxic lymphocyte maturation factor (interleukin 12) is a synergistic growth factor for hematopoietic stem cells. J Exp Med 178:413, 1993.
12. Jacobsen SEW, Okkenhaug C, Myklebust J, Veiby OP, Lyman SD: The Flt3-ligand potently and directly stimulates the growth and expansion of primitive murine bone marrow progenitor cells in vitro: Synergistic interactions with interleukin (IL) 11, IL-12, and other hematopoietic growth factors. J Exp Med 181:1357, 1995.
13. Dybedal I, Larsen S, Jacobsen SEW: IL-12 directly enhances in vitro murine erythropoiesis in combination with IL-4 and stem cell factor. J Immunol 154:4950, 1995.
14. Ploemacher RE, Van Soest PL, Boudewijn A, Neben S: Interleukin-12 enhances interleukin-3 dependent multilineage hematopoietic colony formation stimulated by interleukin-11 or steel factor. Leukemia 7: 1374, 1993.
15. Ploemacher RE, Van Soest PL, Voorwinden H, Boudewijn A: Interleukin-12 synergizes with interleukin-3 and steel factor to enhance recovery of murine hematopoietic stem cells in liquid culture. Leukemia 7:1381, 1993.
16. Jackson JD, Yan Yun, Brunda MJ, Kelsey LS, Talmadge JE: Interleukin-12 enhances peripheral hematopoiesis in vivo. Blood 85:2371, 1995.
17. Bellone G, Trinchieri G: Dual stimulatory and inhibitory effect of NK cell stimulatory factor/IL-12 on human hematopoiesis. J Immunol 153: 930, 1994.
18. Fardoun-Joalland D, Teixeira-Lebrun G, Lenormand B, Dzondo-Gadet M, Vannier JP: Synergism of interleukin-12 and interleukin-3 on development of hematopoietic progenitors. Eur J Haematol 54:172, 1995.
19. Bertolini F, Soligo D, Lazzari L, Corsini C, Servida F, Sirchia G: The effect of interleukin-12 in ex-vivo expansion of human haemopoietic progenitors. Br J Haematol 90:935, 1995.
20. Fardoun-Joalland D, Teixeira-Lebrun G, Jouan-Beades F, Lenormand B, Dzondo-Gadet M, Vannier JP: Sensitivity of myeloid progenitors to NK-cell depletion. Am J Hematol 47:290, 1994.